

On inter-species regression analysis

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Abstract

When conducting inter-species regression analyses, the phylogenetic relationships between the individual species need to be taken into account. In this paper, a procedure for conducting such analyses is discussed, which only requires the use of a measure of relationship between pairs of species, rather than a complete phylogeny, and which at the same time assesses the importance to be attached to the relationships with regard to the conclusions reached. The procedure is applied to data from Minder *et al.* (2005), relating testis size to mean hind tibia length, duct length and spermathecal area in 15 species of Scathophagidae (Diptera). We show that considering the phylogenetic structure significantly improves the fit of the model to the data. We find a robust relationship between testis size and spermathecal area but could not support one between testis size and spermathecal duct length.

Keywords: likelihood based inference, Ornstein-Uhlenbeck process, phylogenetic relationship.

1 Introduction

Comparative studies are a widely employed and powerful tool in evolutionary investigations. They have been used to elucidate macro-evolutionary patterns for many phenomena, including testis and sperm size evolution (e.g. Gage 1994; Hosken 1997), brain size evolution (e.g. Martin 1981; Pagel & Harvey 1989) and the scaling of metabolic rates (e.g. Thompson & Withers 1998; McNab 2002). Formerly, species values of characters of interest were regressed against putative predictor variables to elucidate possible relationships (e.g. Cummins & Woodall 1985). However, because of common descent, species do not represent independent data points, and hence species level analyses based on simple regression analyses have been criticised (Harvey & Pagel 1991). This is not to say that phylogeny has primacy of cause over other factors, merely that species level analyses may be misleading (Harvey 2000). For example, a simulation study by Martins & Garland (1991) investigated the across-species association between two variables, and found that the Type I error rate was 16%; when they employed phylogenetic control, the error in the regression was reduced to 5%. This paper presents a procedure for conducting regression analyses in the presence of phylogenetic relationships, which also assesses the importance of these relationships in the analysis. The procedure is based on the Ornstein–Uhlenbeck model of the way in which inter-species differences evolve, and is closely related to the simplest version of Hansen’s (1997) approach.

In order to derive our procedure, we begin with a more detailed exposition of the underlying problem. In the classical regression model, the value y of the ‘dependent’ variable of interest is expressed linearly in terms of the values $x^{(1)}, x^{(2)}, \dots$ of a number of explanatory ‘covariables’, up to an additive ‘error’ e , which accounts for any variation in y not attributable to the covariables. Thus, for each of n observations indexed by i , $1 \leq i \leq n$, we write

$$y_i = \beta^{(0)} + \beta^{(1)}x_i^{(1)} + \beta^{(2)}x_i^{(2)} + \dots + \beta^{(k)}x_i^{(k)} + e_i, \quad (1)$$

where the $\beta^{(j)}$, $0 \leq j \leq k$, are the coefficients which relate the values of the covariables to that of y , and the e_i are needed because, in practice, it is usually impossible to find values $\beta^{(0)}, \beta^{(1)}, \dots, \beta^{(k)}$ such that

$$y_i = \beta^{(0)} + \beta^{(1)}x_i^{(1)} + \beta^{(2)}x_i^{(2)} + \dots + \beta^{(k)}x_i^{(k)} \quad (2)$$

is *exactly* true for all i , if $n \geq k + 2$. The values of the parameters $\beta^{(j)}$ are estimated and their significance tested with reference to some probabilistic model, which is assumed to govern the values e_i of the errors actually occurring; the simplest assumption is that the e_i , $1 \leq i \leq n$, arise as realizations of *independent* random variables ε_i , $1 \leq i \leq n$, which have zero mean and common unknown variance σ^2 , and are normally distributed.

If the indices $1 \leq i \leq n$ in fact represent n species, as in the setting introduced above, and if the measured values y_i and $x_i^{(1)}, \dots, x_i^{(k)}$ are ‘typical’ values for the

species, the assumption of independent errors may well be violated. This is because the variation in y unexplained by the $x^{(j)}$ can be thought of in part as resulting from evolutionary change in other, unobserved explanatory covariables, so that closely related species can be expected to exhibit rather similar values of e . Hence, when conducting regression analyses with such data, it seems important to take the phylogeny into account (Harvey & Pagel, 1991).

The reasons for doing so are quite simple, and have long been understood in the context of quite general regression models. When the errors ε_i in such a model are in fact correlated, ordinary least squares (OLS) procedures still give parameter estimates which have the right expectation and are asymptotically consistent. However, as is especially relevant when the number of species is fixed and perhaps not large, their precision is less than that of the best estimates possible for the actual correlation structure [Draper & Smith (1966: 80)]. Moreover, estimates of the precision of the OLS estimates, calculated in accordance with OLS assumptions, may be seriously in error [see, for example, Scheffé (1959: 339–343 and §10.4)]. In such cases, significance tests based on OLS are dangerous. In the particular context of inter-species regression, these features of the OLS analysis were noted by Pagel (1993).

There is nonetheless still some debate about the efficacy of such phylogenetic control. This is primarily because the covariance of traits is explained by ecology and phylogeny, which typically overlap; hence, by controlling for phylogeny, variance due to ecology is also removed because of the overlap (McNab 2002). Despite such arguments, most investigators today use some form of phylogenetic control.

A number of methods have been proposed for incorporating phylogeny into the regression, including trait mapping (Ridley 1985), nested analysis of variance (Bell 1989, Stearns 1992), pairwise comparison (Felsenstein 1985, Møller & Birkhead 1992), independent contrasts (Felsenstein 1985), and more directly Ornstein–Uhlenbeck based analyses (Hansen & Martins 1996): see also Lynch (1991) and Freckleton, Harvey & Pagel (2002). These methods all involve the use of a pre-existing phylogeny. Here, we propose a simple and effective procedure, which can either be applied using a known phylogeny, or else just using an inter-species distance matrix from which a phylogeny could potentially be constructed. The procedure has the advantage that it not only respects the phylogeny, but also allows one to gauge its importance in the analysis. It also takes proper account of the (unknown) value at the root of the phylogeny.

2 Procedure

The basic idea is to return to the underlying assumption, that the e_i 's result from a process of evolution along the branches of the phylogenetic tree. The evolutionary model that we use, which can be thought of as a natural generaliza-

tion of that of independent, normally distributed errors with common variance, supposes that the ‘error’ component evolves along each branch of the phylogenetic tree as an Ornstein–Uhlenbeck (O-U) process, as discussed in some detail in Felsenstein (1988) and in Hansen & Martins (1996). The O-U process looks locally in time like a Brownian motion, as would naturally be the result of many small random genetic changes; however, it also has a tendency to return towards zero, which can be thought of as the result of selective pressure acting against departures from equality in (2), the strength of the tendency being larger for larger departures. For our purposes, the main features of the process are that it is a time-reversible Markov process, that its values are normally distributed, and that its autocorrelations decay exponentially with elapsed time. It is also important in acting as a good approximation to a wide variety of processes that result from the combination of random disturbances with a tendency to move back towards zero, in much the same way that the normal distribution is frequently a good approximation to sums of weakly dependent random variables. It thus represents a plausible null model for describing the ‘errors’ arising during evolution: see, for instance, Lande (1976). Its distributions are entirely characterized by the diffusion constant (infinitesimal variance) τ^2 of its locally Brownian behaviour and by the exponential decay rate λ of correlations; its equilibrium distribution is normal, with mean zero and variance $\sigma^2 = \tau^2/(2\lambda)$. We shall denote such a process by $\text{OU}(\tau^2, \lambda)$.

Our model supposes that an $\text{OU}(\tau^2, \lambda)$ process starts in equilibrium at the root of the phylogenetic tree, and runs, with time corresponding to distance along the branch, until the first split. At this point, its value is taken as the initial value for two *independent* $\text{OU}(\tau^2, \lambda)$ processes, which then continue to run along the two branches until they split again; and so on. The species, the leaves of the trees, are assigned as values of e_i the values of the $\text{OU}(\tau^2, \lambda)$ –processes at the ends of the final n branches. This model of evolution along the tree results in values e_i realized from jointly normally distributed random variables $\varepsilon_1, \dots, \varepsilon_n$ having equal variances σ^2 , but now with correlations

$$C_{il}(\lambda) := \text{Corr}(\varepsilon_i, \varepsilon_l) = e^{-\lambda d_{il}}, \quad (3)$$

where d_{il} is the distance between species i and species l along the tree; see, for example, Hansen & Martins (1996: Equation (7)). Details are given in the Appendix.

The value of the decay rate λ , in combination with the values of the d_{il} , is seen from (3) to determine the importance of the inter-species correlations in the analysis. However, the dependence between the errors is better understood from the following explicit representation. If two species i and l diverge at a time at which the value of the $\text{OU}(\tau^2, \lambda)$ process for their common ancestral species takes the value X_0 , and if the evolutionary distances to i and l from this time until the present are d_i and d_l , respectively, then the values of the O-U processes

$X^{(i)}$ and $X^{(l)}$ taken by the species i and l can be written as

$$\begin{aligned} X^{(i)} &= X_0 e^{-\lambda d_i} + V^{(i)}; \\ X^{(l)} &= X_0 e^{-\lambda d_l} + V^{(l)}. \end{aligned} \tag{4}$$

Here, X_0 , $V^{(i)}$ and $V^{(l)}$ are *independent*, and the latter two random variables have zero means and variances

$$\frac{\tau^2}{2\lambda}(1 - e^{-2\lambda d_i}) \quad \text{and} \quad \frac{\tau^2}{2\lambda}(1 - e^{-2\lambda d_l}),$$

respectively. The correlation between $X^{(i)}$ and $X^{(l)}$ arises solely as a result of the elements containing X_0 , the value at their common ancestral species, and these elements can be seen to decline at exponential rate λ as evolutionary distance increases. In the extreme in which $\lambda \rightarrow 0$, the elements containing X_0 both remain constant at the value X_0 , and the Brownian diffusion model with diffusion constant τ^2 results. In the extreme in which $\lambda \rightarrow \infty$, the elements containing X_0 both tend to zero, and the values $X^{(i)}$ and $X^{(l)}$ become independent, implying independent errors for each species. Thus, when fitting the OU(τ^2, λ) model to data, a best fit with large λ indicates more or less independent species, and one with λ very small indicates a Brownian-like model of evolution, a fact noted also by Blomberg *et al.* (2003) (with their parameter d corresponding to our $e^{-\lambda}$). Neither limit is, however, entirely free of surprises: see the Appendix §4.2 for more details.

Regression analysis based on this model is easy if the phylogenetic tree — specifically, all the tree-distances d_{il} between pairs of species — are known. Then, for any fixed λ , $0 < \lambda < \infty$, the problem reduces to a generalized least squares analysis: the correlation matrix $C(\lambda)$ can be calculated, and maximum likelihood for the linear model with normally distributed errors and known correlation matrix can be used to find estimates $\hat{\beta}^{(0)}(\lambda), \hat{\beta}^{(1)}(\lambda), \dots, \hat{\beta}^{(k)}(\lambda)$ and $\hat{\sigma}^2(\lambda)$ of the remaining model parameters, together with $L(\lambda)$, the maximum value of the log-likelihood for this value of λ . The value $\hat{\lambda}$ to be used as an estimate of the true value of λ is now obtained by maximizing $L(\lambda)$ iteratively with respect to λ , for instance using a golden section search. This, as in Hansen (1997: 1345), yields the final parameter estimates

$$\hat{\lambda}, \hat{\beta}^{(0)}(\hat{\lambda}), \dots, \hat{\beta}^{(k)}(\hat{\lambda}), \hat{\sigma}^2(\hat{\lambda})$$

for the regression.

This rather simple procedure has an important drawback. If, for fixed τ^2 , the value of λ becomes very small, the variance $\sigma^2 = \tau^2/(2\lambda)$ of the equilibrium distribution becomes large, and, as a result, any particular observed value has correspondingly low likelihood. Indeed, for such λ , all values are strongly related to the (unknown) value at the root, which is itself a single value chosen from the

equilibrium distribution. This leads to a large negative contribution to the log likelihood, reflecting nothing more than the potential variability in the value at the root, whose effects are clearly visible in Hansen (1997: Tables 1 and 2). It may seem unnatural to include this in a comparative study, in which the value of the overall mean $\beta^{(0)}$ is typically of little interest. There is also a companion, biological consideration; for very small λ , it is doubtful whether enough time can have elapsed for the value at the root to have reached statistical equilibrium. In view of this, we prefer to centre all the covariables $x^{(1)}, \dots, x^{(k)}$ at zero, and to base the analysis on the likelihood derived from the joint distribution of the *centred* y -values

$$y_1 - \bar{y}, \dots, y_n - \bar{y},$$

where \bar{y} , as usual, denotes the overall mean of the observations. Because the covariables have been centred, the model now becomes

$$y_i - \bar{y} = \beta^{(1)}x_i^{(1)} + \beta^{(2)}x_i^{(2)} + \dots + \beta^{(k)}x_i^{(k)} + \tilde{\varepsilon}_i, \quad (5)$$

where $\tilde{\varepsilon}_i = \varepsilon_i - \bar{\varepsilon}$. The overall mean $\beta^{(0)}$ no longer appears in the model, all other parameters have their original meaning, and the log likelihood no longer converges to $-\infty$ as $\lambda \rightarrow 0$, but instead approaches that of the Brownian model. Other attempts to circumvent this difficulty, used in Blomberg *et al.* (2003) and in Butler & King (2004), are discussed in the Appendix, at the end of §4.1; neither seems to be entirely satisfactory.

For each given λ , the linear model theory also gives the standard deviations to be associated with the parameter estimates $\hat{\beta}^{(j)}(\lambda)$, $0 \leq j \leq k$, and these can be used with $\lambda = \hat{\lambda}$ as reasonable approximations to the standard deviations of the estimates $\hat{\beta}^{(j)}(\hat{\lambda})$, and hence for tests of hypotheses. However, $\hat{\lambda}$ has been chosen from the data, and this source of variability is not included in such ‘plug-in’ approximations; simulating data samples from the model obtained from the estimated parameters, and then using an identical estimation procedure, gives an alternative way of judging the actual precision obtained, as well as indicating any possible bias. If the value of λ is itself of interest, an approximate 95% confidence region based on large sample theory is given by the set of all λ such that

$$L(\hat{\lambda}) - L(\lambda) \leq 2 \quad (6)$$

[c.f. Edwards (1972: 80), Hansen (1997: 1345)]. This region may include $\lambda = \infty$, in which case an analysis that neglects inter-species correlations should still be reliable. Again, simulating data samples from the estimated model gives another measure of the variability in the estimates of λ .

In practice, the phylogenetic tree is never known precisely, complete with distances. However, the method proposed here can be expected to give useful results, even when the distances d_{il} are only approximately known; as long as the correlation structure is reasonably represented, gross errors in the conclusions

arising from this source should be avoided. Thus, if any molecular or morphological data for the species are available, on the basis of which a tree can be reconstructed, this can be carried out, and the corresponding tree distances used for the d_{il} .

Alternatively, this relatively difficult step can be avoided by using the morphological and molecular data to define a measure of distance between pairs of species — in any case, often the starting point for a tree construction — and by then using these ‘raw’ distances directly in place of the d_{il} . This procedure may seem controversial, but it should not be. If the raw distances are rather close to being tree distances, then the tree constructed from them should yield inter-species distances which are not very different, and the results of the procedure will change correspondingly little. However, if the raw distances are not particularly tree-like, then the tree constructed from them may well yield rather different inter-species distances, but without any guarantee that they result in a more reliable picture of the actual correlations. Computationally feasible tree growing algorithms provide intelligent heuristics, but offer no guarantee of finding the correct phylogeny. However, using the raw distances, one at least has tangible data as input, rather than output from a black box, and our procedure, because of the freedom to choose the value of λ , still gives a reasonable idea of how strongly relationship (expressed in terms of the raw distances) affects correlation. The phylogeny may not have been determined, but the analysis still makes reasonable allowance for inter-species similarity.

In theory, there may be a problem if the raw distances are too far from being tree distances, because the resulting matrices $C(\lambda)$ need not then be positive semi-definite for all values of λ , as has to be the case for correlation matrices. However, Bochner’s theorem [Defant & Floret (1993: 316)] implies that p ’th powers of l_2 -distances, for $p \leq 2$, never give rise to this problem, and that the same applies if a distance can be represented as a sum of such distances; thus, for instance, Hamming distance (number of mismatches) can be used for molecular data, and can be added to Euclidean distances between morphometrical characters.

Computer programs, written in R, for performing both estimation and simulation from the estimated model, can be obtained from the authors.

3 Example

The procedure is illustrated by application to data in Minder *et al.* (2005), with a regression of testis size y as a function of mean hind tibia length (HTL) $x^{(1)}$, spermathecal duct length $x^{(2)}$ and spermathecal area $x^{(3)}$ in 15 species of Scathophagidae (Diptera; true flies) [Table 1]. In the paper above, a corresponding analysis was made using the comparative analysis by independent contrasts program (CAIC) (Purvis & Rambaut 1994) to correct for the phylogeny, which was de-

duced from that of Bernasconi *et al.* (2000), itself derived from inter-specific differences in the sequence of 810 mDNA letters coding for the COI gene. Here, we look only at the 15 species considered by Minder *et al.* (2005).

We consider three evolutionary distance matrices d . The first, $d^{(1)}$, is derived from the phylogeny depicted in Bernasconi *et al.* (2000: Fig. 1, 313), with the distance $d_{il}^{(1)}$ between species i and l represented by the level in the tree at which their phylogenies merge (leaves at level 0, nearest neighbours at distance 1, etc.). This tree was rather carefully constructed from the COI data, using information about the positions of codons relative to the reading frame, A–T richness, and so on. Our second evolutionary distance matrix $d^{(2)}$ is much cruder, being based solely on the numbers of mismatches $d_{il}^{(0)}$ between the COI sequences for species i and l [Table 2]: we set

$$d_{il}^{(2)} = -\log(1 - d_{il}^{(0)}/105). \quad (7)$$

This matrix is chosen merely to reflect the fact that, as with most evolutionary models, the proportion of mismatches converges to a limit exponentially fast as evolutionary distance increases. Here, it is assumed that the limiting proportion of mismatches is 105/810, which is probably rather small in the context, but serves to exaggerate any effect caused by the non-linearity of the proportion of mismatches as a function of time. The third matrix $d^{(3)}$ that we consider is that of the model in which the errors are independent and identically distributed, so that $d_{il}^{(3)} = \infty$ for all $i \neq l$; this, however, can be obtained also as the limit as $\lambda \rightarrow \infty$ of the two previous models.

The procedure described in Section 2, with the correlation matrix $C(\lambda)$ calculated for each given value of λ by substituting the evolutionary distance matrix $d^{(1)}$ into (3), shows that $x^{(2)}$, spermathecal duct length, has no appreciable influence on y . Leaving out this covariable, the log-likelihood is maximized at a value of 27.17, with $\hat{\lambda} = 0.0714$ and with structural parameter estimates

$$\hat{\beta}^{(1)} = 0.2353 \quad \text{and} \quad \hat{\beta}^{(3)} = 24.13, \quad (8)$$

and with $\hat{\tau}^2 = 0.00912$. The standard deviations of $\hat{\beta}^{(1)}$ and $\hat{\beta}^{(3)}$, as calculated from $C(\hat{\lambda})$ and $\hat{\tau}^2$, are 0.078 and 8.05 respectively, and their estimated correlation is -0.294 (cf. Younger (1985: Sections 11.5–11.8)). The approximate 95% confidence interval for λ calculated according to (6) was $[0, 0.6]$.

We describe the variability and dependence implied by the estimated model by first evaluating a quantity MSD, the median over all pairs of species i and l of

$$\text{SD}(i, l) := \sqrt{\mathbb{E}\{(\varepsilon_i - \varepsilon_l)^2\}} = \frac{\hat{\tau}}{\sqrt{\hat{\lambda}}} \sqrt{(1 - e^{-\hat{\lambda}d_{il}})},$$

the standard deviation of the difference between the errors for species i and l , as calculated for the estimated model (see (12) below). MSD is thus a measure of

the typical variability to be expected in such differences. We then consider the values of

$$\text{RSD}(i, l) := \text{SD}(i, l) / \text{MSD}.$$

If dependence has little effect on the analysis (λ large), then all such values are close to 1; if a pair (i, l) is strongly dependent, then $\text{RSD}(i, l)$ is close to zero. For the analysis here, we consider a pair (i_1, l_1) (*Norellia striolata* and *N. spinimona*) which are very closely related, and another, (i_2, l_2) (*Scathophaga sulli* and *S. furcata*), which are moderately closely related, as examples. For the analysis just conducted, we find that

$$\text{MSD} = 0.2711, \quad \text{RSD}(i_1, l_1) = 0.3460 \quad \text{and} \quad \text{RSD}(i_2, l_2) = 0.7002.$$

The maxima of the log-likelihood for the submodels obtained by omitting either $x^{(1)}$ or $x^{(3)}$ are both substantially more than 2 smaller than that obtained above (differences 3.47 and 3.24 respectively), indicating that, at the 5% level, neither submodel should be preferred to the model estimated above. This means that hind tibia length (HTL) and spermathecal area are associated with testis size across the Scathophagidae after phylogenetic control using $d^{(1)}$. Minder *et al.* (2005) concluded that testis size was related to spermathecal area and spermathecal duct length, but not with HTL, after phylogenetic control. Our analysis supports the relationship with spermathecal area, making this a robust conclusion; especially since Minder *et al.* (2005) also found it with a species comparison. The relationship with HTL is intuitively appealing, as the simplest expectation is that, when a species gets larger, so do all of its body parts. Since the testis area to spermathecal duct length relationship is not robust in the different analyses, some caution must be exercised when considering the relationship reported in Minder *et al.* (2005).

In order to judge the validity of the procedure, and to obtain an alternative assessment of the variability in the estimates, the model (8) and the correlation structure $C(\hat{\lambda})$ for the errors were held fixed, and data from this model distribution were simulated 1'000 times. The estimation procedure was then applied individually to each of the 1'000 resulting sets of data. These led to mean values

$$\bar{\beta}^{(1)} = 0.2353, \quad \text{and} \quad \bar{\beta}^{(3)} = 24.14 \tag{9}$$

for the structural parameter estimates, with empirical standard deviations of 0.0855 and 8.00 and an empirical correlation of -0.201 for the 1'000 estimates of $\beta^{(1)}$ and $\beta^{(3)}$. The mean values $\bar{\beta}^{(1)}$ and $\bar{\beta}^{(3)}$ in (9) are well in accord with the regression parameters $\hat{\beta}^{(1)}$ and $\hat{\beta}^{(3)}$ of the model (8), as are the empirical standard deviations of the estimates $\hat{\beta}^{(1)}$ and $\hat{\beta}^{(3)}$ with the values calculated from $C(\hat{\lambda})$ and $\hat{\tau}^2$.

The estimates of variability and dependence in the simulated data were by no means as stable. This is not particularly surprising, in view of the small number (15) and large variability ($\text{MSD} = 0.2711$, as compared with estimated effects

$\hat{\beta}^{(1)} \times \text{SD}(x^{(1)}) = 0.0912$ and $\hat{\beta}^{(3)} \times \text{SD}(x^{(3)}) = 0.0524$) of the observations. In just over 40% of the simulations, λ was estimated to be zero, and in a further 5% to be infinity; the median value was 0.05, close to the actual value 0.0714, and the 90% confidence interval was $[0, 0.69]$. For the quantity MSD, the empirical mean over the 1'000 simulations was 0.2303, rather lower than the true value of 0.2711, and the empirical standard deviation was 0.0591. The negative bias in the empirical mean is to be expected, just as in the classical model with independent errors, where maximum likelihood makes no allowance for the fitted degrees of freedom when estimating the standard deviation. The empirical standard deviation of the MSD values is very much what would be expected when estimating a standard deviation from only 15 observations. The values of $\text{RSD}(i_1, l_1)$ had a minimum value of 0.2966, taken when λ was estimated to be zero, and were thus heavily skewed; the median was 0.3288, not far from the true value of 0.3460, and its 90th percentile was at 0.7000. Analogously, the values of $\text{RSD}(i_2, l_2)$ had a minimum of 0.6567, a median of 0.7002, to be compared with the true value of 0.7223, and a 90th percentile at 0.9833. Thus, despite the wide variability in the estimated values of λ , the results of the simulations as regards variability and dependence still showed a reasonable consistency.

The same analyses can also be conducted with correlations based on the distance matrix $d^{(2)}$. The results are broadly the same; the covariable $x^{(2)}$ is immediately dropped, and the model with $x^{(1)}$ and $x^{(3)}$ has likelihood more than 2 larger than that of either of the models with just one covariable. The parameter estimates in this model are

$$\hat{\beta}^{(1)} = 0.2068 \quad \text{and} \quad \hat{\beta}^{(3)} = 24.11,$$

with estimated standard errors of 0.0929 and 8.13, respectively, all of which are reasonably consistent with (8). For the estimated error structure, we have

$$\text{MSD} = 0.2558, \quad \text{RSD}(i_1, l_1) = 0.3947 \quad \text{and} \quad \text{RSD}(i_2, l_2) = 0.8056,$$

the last two values indicating rather weaker evolutionary dependence than that found using $d^{(1)}$, but not outstandingly so. The main differences between the results with $d^{(1)}$ and $d^{(2)}$ are that the maximum of the log likelihood using $d^{(2)}$ (at 25.57) is smaller by 1.6 than that for $d^{(1)}$, suggesting that using the cruder matrix $d^{(2)}$ leads to a somewhat less good fit, and that the decision to keep $x^{(1)}$ in the model is based on a likelihood difference of 2.12, quite a bit smaller than that obtained using $d^{(1)}$, indicating that the less good fit indeed entails some loss of precision, though not enough to change any of the main conclusions. The results of simulations confirmed the reliability of the procedure using $d^{(2)}$ in much the same way as it did when using $d^{(1)}$.

If phylogenetic correlation is entirely neglected, and the model with independent and identically distributed errors is used, a significant loss of precision

is observed. The variable $x^{(2)}$ is still immediately rejected, and the structural parameters are estimated by

$$\hat{\beta}^{(1)} = 0.2065 \quad \text{and} \quad \hat{\beta}^{(3)} = 19.82,$$

fairly much as before; variability is estimated by $\text{MSD} = 0.2674$ (here corresponding to a residual standard deviation of around 0.19), and all RSD-values are 1. This model, however, has a log likelihood of only 23.53, more than 2 smaller than either of the two models fitted using phylogenetic correlation, and would therefore be rejected in comparison to them. Furthermore, under independence, the model with just $x^{(3)}$ has log likelihood 22.42, only 1.1 smaller, corresponding to a two-sided P-value of about 14%. This might suggest that $x^{(1)}$ should also be omitted from the model; however, the alternative for the effect of $x^{(1)}$ is clear and one-sided, and the relevant P-value is more properly about 7%, giving weak support for retaining $x^{(1)}$ in the model. Nonetheless, if phylogenetic correlation were neglected, there could be a danger that the effect of HTL on testis size would be missed.

4 Discussion

The method that we propose for conducting inter-species regression analyses is developed from the engagingly simple idea of using likelihood-based methods in conjunction with a stationary Ornstein-Uhlenbeck model of evolution. The result is a procedure which can be carried out without knowing the complete phylogeny — a measure of the evolutionary distance between pairs of species suffices — and which, at the same time, assesses the importance of inter-species relationship for the analysis. It is thus rather surprising that these advantages have not been emphasized earlier.

Our example illustrates that the method works much as expected when applied to a ‘typical’ biological data set. Although the detailed correlation structure was not reliably estimable, because there were few data points and a low signal to noise ratio, this still did not prevent the regression coefficients and their precisions being successfully estimated. Indeed, the procedure performed very well in a number of respects. It estimated the regression parameters and the variability of these estimates satisfactorily; it highlighted the extent to which the highly variable data did not support accurate estimation of the underlying dependence structure; and it indicated that, with these data, replacing the phylogeny with a crude estimate of the inter-species distances had little impact on the final conclusions.

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Appendix

4.1 The O-U model.

The O-U model for the error structure can be constructed as follows. We begin with a phylogeny consisting of a tree T with a root 0, n leaves and a set E of edges. The length of an edge e is denoted by $\ell(e)$, and the distance from its rootward node to the root by $t(e)$. There is a unique path $P_i = (e_{i1}, e_{i2}, \dots, e_{im_i})$ from the root to each leaf i in T , $1 \leq i \leq n$, with $0 = t(e_{i1}) < t(e_{i2}) < \dots < t(e_{im_i})$, with $t(e_{ij}) = t(e_{i,j-1}) + \ell(e_{i,j-1})$ for each $2 \leq j \leq m_i$, and with leaf i at distance $d_{0i} = t(e_{im_i}) + \ell(e_{im_i})$ from the root.

An OU (τ^2, λ) -process X has the property that, for any $s, u > 0$, the conditional distribution of $X(s+u)$ given $X(s) = x$ is that of $xe^{-\lambda u} + X_0(u)$, where X_0 is an OU (τ^2, λ) -process with $X_0(0) = 0$, and thus $X_0(u)$ is normally distributed with mean 0 and variance $\sigma^2(1 - e^{-2\lambda u})$, where we write

$$\sigma^2 := \tau^2 / (2\lambda)$$

for the equilibrium variance. Hence the O-U error model on the tree can be constructed by associating with each edge e an *independent* normally distributed random variable $Z(e)$ with mean 0 and variance $\sigma^2(1 - e^{-2\lambda\ell(e)})$, and by then defining the error at leaf i to be

$$\varepsilon_i := Z^{(0)}e^{-\lambda d_{0i}} + \sum_{j=1}^{m_i} Z(e_{ij})e^{-\lambda(d_{0i}-t(e_{ij})-\ell(e_{ij}))}, \quad (10)$$

where $Z^{(0)}$ denotes the value of the error at the root. In our formulation, in which the O-U process stationary, $Z^{(0)}$ is an independent normal random variable with mean 0 and variance σ^2 .

It is now simple to deduce from (10) and from the independence of the $Z(e)$'s that $\mathbb{E}\varepsilon_i = 0$ for all i and that (as has to be, because of stationarity)

$$\begin{aligned} \text{Var } \varepsilon_i &= e^{-2\lambda d_{0i}} \text{Var } \{Z^{(0)}\} + \sum_{j=1}^{m_i} \text{Var } \{Z(e_{ij})\} e^{-2\lambda(d_{0i}-t(e_{ij})-\ell(e_{ij}))} \\ &= \sigma^2 e^{-2\lambda d_{0i}} \left\{ 1 + \sum_{j=1}^{m_i} \{1 - e^{-2\lambda\ell(e_{ij})}\} e^{2\lambda(t(e_{ij})+\ell(e_{ij}))} \right\} \\ &= \sigma^2, \end{aligned}$$

since the sum telescopes because $t(e_{ij}) = t(e_{i,j-1}) + \ell(e_{i,j-1})$, and since $t(e_{i1}) = 0$ and $d_{0i} = t(e_{im_i}) + \ell(e_{im_i})$. For the covariances, we similarly have

$$\text{Cov } (\varepsilon_i, \varepsilon_l) = e^{-\lambda(d_{0i}+d_{0l})} \text{Var } \{Z^{(0)}\} + \sum_{j=1}^{m_{il}} \text{Var } \{Z(e_{ij})\} e^{-\lambda(d_{0i}+d_{0l}-2\{t(e_{ij})+\ell(e_{ij})\})},$$

where

$$P_i \cap P_l = (e_{i1}, \dots, e_{im_{il}}) = (e_{l1}, \dots, e_{lm_{il}})$$

is the overlap between the paths leading from the root to i and l . Hence

$$\begin{aligned} \text{Cov}(\varepsilon_i, \varepsilon_l) &= e^{-\lambda(d_{0i}+d_{0l})} \left\{ 1 + \sum_{j=1}^{m_{il}} \{1 - e^{-2\lambda\ell(e_{ij})}\} e^{2\lambda(t(e_{ij})+\ell(e_{ij}))} \right\} \\ &= \sigma^2 e^{-\lambda\{(d_{0i}-t(e_{im_{il}})-\ell(e_{im_{il}}))+(d_{0l}-t(e_{lm_{il}})-\ell(e_{lm_{il}}))\}} \\ &= \sigma^2 e^{-\lambda(d_i+d_l)} = \sigma^2 e^{-\lambda d_{il}}, \end{aligned} \tag{11}$$

where

$$d_i = d_{0i} - t(e_{im_{il}}) - \ell(e_{im_{il}}) \quad \text{and} \quad d_l = d_{0l} - t(e_{lm_{il}}) - \ell(e_{lm_{il}}),$$

and $d_{il} = d_i + d_l$ is the tree-distance from i to l , since $e_{im_{il}} = e_{lm_{il}}$ and this is the last common edge in the paths P_i and P_l . Equation (3) now follows immediately.

There is a non-trivial limit as $\lambda \rightarrow 0$. In this limit, each $Z(e)$ is normally distributed with mean 0 and variance $\ell(e)\tau^2$, so that the joint *conditional* distribution of the ε_i 's given any fixed value z_0 of $Z^{(0)}$ is the same as for the Brownian model of evolution with infinitesimal variance τ^2 and having value z_0 at the root. Equivalently, one can check that the covariance structure of the OU (τ^2, λ) model, restricted to the space of linear combinations of $\varepsilon_i - \bar{\varepsilon}$, $1 \leq i \leq n$, converges to that of the Brownian model on the same space, where $\bar{\varepsilon} := n^{-1} \sum_{i=1}^n \varepsilon_i$, irrespective of the value of z_0 . Note that this limiting model does *not* have an equilibrium distribution.

Instead of taking $Z^{(0)}$ to have the stationary distribution in the O-U model, Blomberg *et al.* (2003) suppose that $\text{Var } Z^{(0)} = 0$, so that $Z^{(0)}$ is considered to be fixed at some (unspecified) value z_0 . Thus their formulae for variances and covariances differ from those above, in that the first term in each sum is lost, giving, in our notation,

$$\begin{aligned} \text{Var}' \varepsilon_i &= \sigma^2(1 - e^{-2\lambda d_{0i}}); \\ \text{Cov}'(\varepsilon_i, \varepsilon_l) &= \sigma^2(e^{-\lambda d_{il}} - e^{-\lambda(d_{0i}+d_{0l})}), \end{aligned}$$

so that the root to leaf distances also enter their formulae. To this added complication comes the problem of the means; they now have $\mathbb{E}'(\varepsilon_i) = z_0 e^{-\lambda d_{0i}}$. Thus the usual linear model analyses, conducted on the assumption that errors have zero mean, are inconsistent with their formulation (at least, if the d_{0i} 's are not all equal and if $0 < \lambda < \infty$) unless z_0 is fixed to be zero. Hence their formulae are in general only valid if a time earlier than the first split in the phylogeny is known, *at which the error is known to be exactly 0*, and if this time is then taken to be the root. This seems to be rather an unlikely circumstance, and there is certainly no way of inferring the value of such a time from an inter-species distance matrix. Hence the stationary O-U model is much to be preferred; it pre-supposes merely

that $\text{OU}(\tau^2, \lambda)$ -style evolution had already been taking place for a reasonable length of time before the first split in the phylogeny.

Butler & King (2004), who are principally interested in more detailed modelling of adaptive evolution, have a very ingenious approach to $Z^{(0)}$. They treat its value as a *parameter* of the model, to be estimated along with τ^2 and λ . This approach again has the disadvantage of including more elements of the phylogeny in the formulae. It is also not clear that inference about $\beta^{(1)}, \dots, \beta^{(k)}$ would remain invariant using their approach, if the root were moved further into the past from the time of the first split in the phylogeny, while leaving the rest of the phylogeny unchanged; this should, however, logically be the case. In view of these considerations, our simpler model would seem to be preferable here also.

4.2 Estimation as a function of λ .

To illustrate how the model estimated from data varies with the choice of λ , consider the case in which there are no covariates, so that $y_i = \mu + \varepsilon_i$. Then the statistic

$$S^2 := \frac{1}{n-1} \sum_{i=1}^n (y_i - \bar{y})^2 = \frac{1}{2n(n-1)} \sum_{i=1}^n \sum_{l=1}^n (y_i - y_l)^2,$$

for a model with independent and identically distributed errors, is a natural estimator of the common variance of the ε_i 's. For the O-U error model, we have

$$\mathbb{E}\{(\varepsilon_i - \varepsilon_l)^2\} = 2\sigma^2(1 - e^{-\lambda d_{il}}) = \frac{\tau^2}{\lambda}(1 - e^{-\lambda d_{il}}), \quad (12)$$

from (11), so that S^2 estimates $\tau^2 D_\lambda$, where

$$D_\lambda := \frac{1}{2n(n-1)} \sum_{i=1}^n \sum_{l=1}^n \frac{1}{\lambda} (1 - e^{-\lambda d_{il}}).$$

Hence, for given λ , a reasonable (but in general not optimal) estimator of τ^2 is given by

$$\hat{\tau}_\lambda^2 := S^2 / D_\lambda. \quad (13)$$

If λ is very large, then $D_\lambda \sim 1/(2\lambda)$, and it thus follows from (13) that S^2 estimates the equilibrium variance $\sigma^2 = \tau^2/(2\lambda)$, as is to be expected close to the model of independent errors. Note, however, that S^2 is a fixed function of the data, so that, from (13), the sequence of models estimated as λ increases has $\hat{\tau}_\lambda^2 \sim 2\lambda S^2$ growing to infinity linearly with λ . Thus, in this limit, the rate of random disturbances estimated from a fixed set of data tends to infinity.

If, on the other hand, $\lambda \rightarrow 0$, then D_λ increases to its maximal value of

$$D_0 = \frac{1}{2n(n-1)} \sum_{i=1}^n \sum_{l=1}^n d_{il},$$

and τ^2 is estimated by S^2/D_0 , as appropriate for the Brownian model.

In the limit as $\lambda \rightarrow 0$, the curiosity is rather the limiting value of 1 for $\text{Corr}(\varepsilon_i, \varepsilon_l)$, as implied by (3). The reason for this is as follows. Suppose that X , V_1 and V_2 are independent random variables, and that $X_1 := X + V_1$, $X_2 := X + V_2$. Then

$$\begin{aligned}\text{Corr}(X_1, X_2) &= \text{Var } X / \sqrt{(\text{Var } X + \text{Var } V_1)(\text{Var } X + \text{Var } V_2)} \\ &= 1 / \sqrt{(1 + \eta_1)(1 + \eta_2)},\end{aligned}$$

where $\eta_j = \text{Var } V_j / \text{Var } X$ for $j = 1, 2$. If now $\text{Var } V_1$ and $\text{Var } V_2$ remain fixed, but $\text{Var } X \rightarrow \infty$, it follows that $\text{Corr}(X_1, X_2) \rightarrow 1$; and this despite the fact that

$$\mathbb{E}\{(X_1 - X_2)^2\} = \{\mathbb{E}V_1 - \mathbb{E}V_2\}^2 + \text{Var } V_1 + \text{Var } V_2$$

remains constant. Comparing this setting with that of (4), it follows that the errors ε_i and ε_l for species i and l have correlation 1, in the limit as $\lambda \rightarrow 0$, only because they inherit the same element X_0 from their common ancestor species, whose variance $\sigma^2 = \tau^2/(2\lambda)$ tends to infinity as $\lambda \rightarrow 0$. In particular, as implied by (12), $\mathbb{E}\{(\varepsilon_i - \varepsilon_l)^2\}$ remains bounded away from zero and infinity as $\lambda \rightarrow 0$, so that the random variability between species does not disappear, even though $\text{Corr}(\varepsilon_i, \varepsilon_j) \rightarrow 1$. Indeed, the expression (12) for $\mathbb{E}\{(\varepsilon_i - \varepsilon_l)^2\}$ provides a consistent basis for comparing variability and strength of dependence across different models, and is used as such in Section 3: c.f. also Mathéron's (1965) variogram.

4.3 Departures from equilibrium.

The analysis proposed in this paper supposes that the underlying O-U error process is in equilibrium. This is not a problem unless, as noted in Section 2, values of λ are considered which are so small that it is doubtful whether equilibrium could ever have been reached. However, the value $\lambda = 0$ (for which there can be no equilibrium) represents the well-tried Brownian model of evolution, and it is therefore reasonable to ask how our procedure behaves for λ very close to 0.

Since we base our analysis only on the centred variables $y_i - \bar{y}$, it is enough to understand what happens for *differences* of errors $\varepsilon_i - \varepsilon_l$ between pairs of species. So suppose that the root value $Z^{(0)}$ in (10) is not at equilibrium, but instead has a normal distribution with mean μ and variance v given by

$$\mu := s \left(\frac{\tau}{\sqrt{2\lambda}} \right) e^{-\lambda D} \quad \text{and} \quad v := \left(\frac{\tau^2}{2\lambda} \right) (1 - e^{-2\lambda D});$$

this represents an initial value for the error in the ancestor species of s standard deviations away from zero, at an epoch D units of evolutionary time prior to the

root. Then it is easy to calculate

$$\begin{aligned}\mathbb{E}(\varepsilon_i - \varepsilon_l) &= \mathbb{E}\{Z^{(0)}(e^{-\lambda d_{0i}} - e^{-\lambda d_{0l}})\} \\ &= s \left(\frac{\tau}{\sqrt{2\lambda}} \right) e^{-\lambda D} e^{-\lambda D_{il}} (1 - e^{-\lambda \delta_{il}}),\end{aligned}$$

where $D_{il} = \min\{d_{0i}, d_{0l}\}$, $\delta_{il} = |d_{0i} - d_{0l}| \leq d_{il}$, and d_{0i} represents the evolutionary time from the root until species i . Hence, writing $\Delta_{il} := D + D_{il}$ for the time from the initial epoch until the i and l ancestries split, we have

$$\mathbb{E}(\varepsilon_i - \varepsilon_l) = s \left(\frac{\tau}{\sqrt{2\lambda}} \right) e^{-\lambda \Delta_{il}} (1 - e^{-\lambda \delta_{il}}), \quad (14)$$

to be compared with the equilibrium standard deviation of $\varepsilon_i - \varepsilon_l$ which, from (12), is given by

$$\text{SD}(i, l) := \left(\frac{\tau}{\sqrt{\lambda}} \right) \sqrt{1 - e^{-\lambda \delta_{il}}}. \quad (15)$$

The ratio of these two quantities is thus

$$\frac{s}{2} \frac{e^{-\lambda \Delta_{il}} (1 - e^{-\lambda \delta_{il}})}{\sqrt{1 - e^{-\lambda \delta_{il}}}} =: \frac{s}{2} r(i, l; \lambda),$$

say, where

$$r(i, l; \lambda) \leq \{e^{-\lambda \Delta_{il}} \sqrt{\lambda \Delta_{il}}\} \sqrt{\delta_{il} / \Delta_{il}}.$$

Thus there is *no mean effect* if the branch lengths to i and l are equal ($\delta_{il} = 0$), or if $\lambda = 0$, or if $\lambda = \infty$, or if $s = 0$; more generally, the effect is small if $\lambda \Delta_{il}$ is either small or large, and the largest effect possible, occurring when $\lambda = 1/(2\Delta_{il})$, is $\frac{s}{2\sqrt{2e}} \sqrt{\delta_{il} / \Delta_{il}}$. Hence, if the *differences* in branch lengths are much smaller than the times from the initial epoch until species diverge, there can be no appreciable mean effect.

For the variability, the conclusions are entirely analogous. It is straightforward to compute

$$\text{Var}\{\varepsilon_i - \varepsilon_l\} - \{\text{SD}(i, l)\}^2 = \left(\frac{\tau^2}{2\lambda} \right) e^{-2\lambda \Delta_{il}} (1 - e^{-\lambda \delta_{il}})^2,$$

and the ratio of this quantity to $\{\text{SD}(i, l)\}^2$, the relative error in the variance induced by assuming the process to be in equilibrium, gives the value $\frac{1}{2}\{r(i, l; \lambda)\}^2$. Once again, there is no correction to be made if the branch lengths to i and l are equal ($\delta_{il} = 0$), or if $\lambda = 0$, or if $\lambda = \infty$; and the largest effect possible is $\frac{1}{4e}\{\delta_{il} / \Delta_{il}\}$. Hence, if the *differences* in branch lengths are much smaller than the times from the initial epoch until species diverge, there can be no appreciable effect on the variances, either.

Table 1: Testis size, mean HTL, duct length and spermathecal size for 15 species of Scathophagidae: data from Minder *et al.* (2005).

Species	Testis size (mm ²) y	mean HTL (mm) $x^{(1)}$	Duct Length (mm) $x^{(2)}$	Spermathecal Area (mm ²) $x^{(3)}$
<i>Cordilura albipes</i>	.169	2.410	.534	.00490
<i>Cleigastrea apicalis</i>	.078	2.080	.412	.00769
<i>Cordilura ciliata</i>	.435	3.290	.604	.01743
<i>Cordilura pubera</i>	.332	2.775	.727	.01611
<i>Microprosopa pallidicauda</i>	.477	2.125	.531	.00795
<i>Norellia liturata</i>	.382	2.095	.962	.02497
<i>Norellia spinimana</i>	.547	2.295	1.086	.02048
<i>Norellia striolata</i>	.855	3.110	1.384	.02397
<i>Phrosia albilabris</i>	.319	2.380	.519	.01485
<i>Scathophaga cineraria</i>	.486	2.750	.561	.01046
<i>Scathophaga furcata</i>	.965	2.430	.541	.02195
<i>Spaziphora hydromyzina</i>	.134	2.000	.235	.01049
<i>Scathophaga stercoraria</i>	.544	2.815	.672	.01044
<i>Scathophaga suilla</i>	.461	2.380	.386	.01002
<i>Scathophaga taeniopa</i>	.699	2.695	.479	.01347

Table 2: Numbers of differing pairs between the sequences of 810 mDNA letters coding for the COI gene in 15 species of Scathophagidae: original sequences from Genbank.

a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	Species
*	70	64	69	72	73	87	83	62	67	75	82	63	65	70	a: <i>Cordilura albipes</i>
	*	92	93	64	69	76	75	81	60	71	74	55	54	61	b: <i>Cleigastra apicalis</i>
		*	63	80	77	88	88	71	73	68	89	71	72	77	c: <i>Cordilura ciliata</i>
			*	81	83	99	96	75	84	88	96	81	81	85	d: <i>Cordilura pubera</i>
				*	66	79	73	82	63	74	55	52	59	63	e: <i>Microprosopa pallidicauda</i>
					*	67	65	77	59	70	70	58	57	63	f: <i>Norellia liturata</i>
						*	9	95	67	80	74	69	72	79	g: <i>Norellia spinimana</i>
							*	94	64	77	71	64	68	75	h: <i>Norellia striolata</i>
								*	69	78	83	68	70	72	i: <i>Phrosia albilabris</i>
									*	36	62	35	20	30	j: <i>Scathophaga cineraria</i>
										*	73	43	41	45	k: <i>Scathophaga furcata</i>
											*	56	56	62	l: <i>Spaziphora hydromyzina</i>
												*	29	38	m: <i>Scathophaga stercoraria</i>
													*	22	n: <i>Scathophaga suilla</i>
														*	o: <i>Scathophaga taeniopa</i>